INJURY TO THE Ca^{++}-TRANSPORTING SYSTEM OF THE SARCOPLASMIC RETICULUM OF THE HEART DUE TO EMOTIONAL AND PAIN-INDUCED STRESS

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Emotional and pain-induced stress (EPS) is known to lead to activation of peroxidation of lipids (POL) in the myocardium and also to disturbance of the contractile function of the heart and to the development of focal contractural injuries to the myocardial cells, which may be prevented by administration of the antioxidant ionol, which inhibits POL [1, 2].

On this basis it was suggested that disturbance of the membrane Ca^{++} transport system by products of POL is an important stage in the pathogenesis of stress injury to the heart. As a result of an excess of Ca^{++}, not removed from the sarcoplasm at the proper time into the sarcoplasmic reticulum (SR), contractural injuries develop and the function of the heart muscle is disturbed [4].

The object of this investigation was to study the effect of EPS on the state of Ca^{++}-transporting system of the SR of heart muscle and the possibility of preventing the disturbances of function of this system by the use of the antioxidant ionol.

EXPERIMENTAL METHOD

Male Wistar rats weighing about 200 g were used. The animals were divided into four groups: 1) control rats; 2) rats subjected to EPS; 3) rats receiving ionol; 4) rats receiving ionol and then subjected to EPS. The rats were decapitated 2 h after the end of exposure to EPS for 5 h. The heart was frozen in liquid nitrogen. To isolate the microsomal fraction the frozen heart was placed in cold medium of the following composition: 0.25 M sucrose, 1 mM EDTA, 3 mM NaN_3, 20 mM Tris-HCl, pH 7, and quickly shredded in a homogenizer of the Politron type (3 × 15 sec). The fraction enriched with membranes of SR fragments was separated by differential centrifugation (14,000-40,000g) with intermediate extraction in 0.6 M KCl. The protein concentration was determined by the biuret method, with the use of sodium deoxycholate.

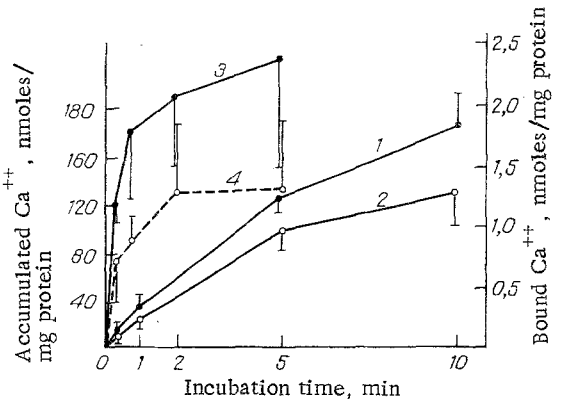

Fig. 1

KEY WORDS: sarcoplasmic reticulum; peroxidation of lipids; stress; myocardium.

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TABLE 1. Indices of Enzyme System for Ca^{++} Ion Transport in SR Membranes (M ± m)

Indices	Control	Ionol	EPS	Ionol + EPS
Activity, nmoles P_{in}/mg protein/min				
Ca, Mg- ATPase	1267±163 (12)	1220±181 (6)	1026±249 (11)	1199±105 (8)
Mg- ATPase	694±113 (12)	688±93 (6)	642±153 (10)	640±187 (8)
Ca- ATPase	502±68 (12)	488±71 (6)	359±127* (10)	469±68 (8)
Velocity, nmoles Ca^{++}/mg protein/min				
of Ca^{++} accumulation	37,2±4,0 (10)	36,2±3,2 (6)	23,1±4,2* (8)	29,0±3,8 (8)
of Ca^{++} binding	3,80±0,42 (10)	4,11±0,85 (6)	2,23±1,14 (8)	3,85±0,31 (8)
Quantity of bound Ca^{++}, nmoles Ca^{++}/mg protein	7,20±2,78 (10)	7,35±3,01 (6)	3,90±1,22 (7)	8,37±3,31 (8)

Legend. 1) Number of experiments given in parentheses; 2) *P < 0.05.

Binding of Ca^{++} with SR membranes was carried out at 25°C in medium of the following composition: 100 mM KCl, 3 mM NaN_3, 9 mM $MgCl_2$, 5 mM ATP, 25 μM $CaCl_2$ (1 μCi $^{45}CaCl_2$), 0.11 mg protein, 20 mM Tris-HCl, pH 7. Samples taken in the course of incubation were filtered through millipore filters (diameter 24 mm, pore size 0.45 μ) and their radioactivity was measured in a scintillation counter. A similar operation was carried out to determine accumulation of Ca^{++} in SR, but in this case the medium contained 5 mM sodium oxalate, 12.5 μM $CaCl_2$ (1 μCi $^{45}CaCl_2$), and 0.05 mg protein. Activity of Ca,Mg-ATPase was determined from the change in pH (within the range 7.00-7.02) in medium of the following composition: 100 mM KCl, 5 mM $MgCl_2$, 5 mM ATP, 12.5 M $CaCl_2$, 0.5 mg protein; 37°C. Mg-ATPase was determined under the same conditions but 0.5 μM EGTA was added instead of $CaCl_2$. Ca-ATPase activity was calculated by subtracting Mg-ATPase activity from Ca,Mg-ATPase activity

EXPERIMENTAL RESULTS

The main indicators of functional activity of the enzyme system for Ca^{++} transport in SR membranes are Ca,Mg-ATPase activity, the rate of Ca^{++} accumulation in the presence of oxalate, and the rate of Ca^{++} binding and quantity of Ca^{++} bound in the absence of oxalate. The first indicator reflects the creation of a transmembrane Ca^{++} ion gradient (active Ca^{++} transport), the last of themreflects maintenance of this gradient.

A graph of accumulation and binding of Ca^{++} in SR membranes is shown in Fig. 1. As a result of EPS both the rate of accumulation and the degree of binding of Ca^{++} clearly were reduced by 25-45%. Values of the initial velocities and activity of cation-dependent ATPases calculated from these results are given in Table 1. They show that Ca-ATPase activity in SR from the hearts of the control rats was 44% of the total Ca,Mg-ATPase activity, falling to 25% after EPS. Practically the whole effect of the decrease in total Ca,Mg-ATPase activity in SR after EPS was thus due to inactivation of Ca-ATPase (responsible for Ca^{++} transport inside SR vesicles), against the background of unchanged Mg-ATPase activity.

However, the efficiency of operation of the enzyme Ca^{++} transport system depends not only on the activity of transport Ca-ATPase, but also on the ability of SR vesicles to retain Ca^{++} ions inside them, i.e., on the permeability of the SR membrane. An increase in membrane permeability inevitably leads to outflow of intravesicular Ca^{++}, whose transport inside the vesicles requires additional activation of Ca-ATPase. If it is accepted that coupling of the work of the Ca pump, i.e., the ratio between the rate of Ca^{++} accumulation and Ca-ATPase activity (Ca/ATP), remains unchanged, the contribution of the increase in permeability to the decrease in the Ca-transporting capacity of SR after EPS can be calculated. For instance, in the control Ca/ATP = 37.2:5.02 = 0.74. By the use of this ratio it is found that after EPS the theoretical value of the rate of accumulation of Ca^{++} is 0.074 × (Ca-ATPase activity during EPS = 359 nmoles P_{in}/mg protein/min) = 26.6 nmoles Ca^{++}/mg protein/min. The difference between this value and that obtained experimentally, i.e., 26.6-23.1 = 3.5 nmoles Ca^{++}/mg protein/min, can be taken to be the rate of leakage of Ca^{++} from the vesicles due to increased permeability of the SR membrane after EPS.

The most likely results of these disturbances may be a decrease in the rate of removal of Ca^{++} from the sarcoplasm and an increase in its concentration therein. In the modern view this important shift, regardless of how caused, leads to the development of what is called the calcium triad, a combination of incomplete relaxation or contractural contraction of the myofibrils, and accumulation of Ca^{++} in the mitochondria and of proteases in the myofibrils [3]. It is this triad which is the most probable basis for foci of necrobiosis and distur-

bances of cardiac function after exposure to EPS.

The results given in Table 1 show that ionol, an inhibitor of POL, did not affect the Ca-transport system in SR of the control animals but prevented the decrease in Ca^{++} accumulation and in Ca-ATPase activity in EPS. This suggests that POL is the main cause of the disturbance of Ca^{++} transport in EPS.

Disturbance of the enzyme system for Ca^{++} ion transport in SR membranes (a decrease in Ca-ATPase activity and an increase in membrane permeability) is due to activation of POL and can be prevented by administration of a POL inhibitor. This means that injury to the membrane system for Ca^{++} transport by POL products is in fact the key stage of stress injury to the heart, and that administration of antioxidants affords prospects for the prevention of such injuries.

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EFFECT OF HYPERBARIC OXYGENATION ON LOCAL TISSUE BLOOD FLOW IN A GRAFT OF SMALL INTESTINE INTENDED FOR ESOPHAGOPLASTY

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Intestinal esophagoplasty is widely used in reconstructive surgery for esophageal obstruction [9, 6]. A serious complication of plastic reconstruction of the esophagus is partial or total necrosis of the graft [4]. These complications are connected with disturbance of the circulation in the graft, the main predisposing causes of which are ligation of some of the mesenteric vessels and the subsequent traumatic manipulations with the graft. To begin with the microcirculation is disturbed, and this largely determines the outcome of the plastic operation and the efficacy of treatment [8]. This makes it clear that one way of improving the viability of an intestinal graft is through correction of the disturbances of its microcirculation.

The method of hyperbaric oxygenation (HBO) has been widely used for the treatment of circulatory disturbances [5]. This method allows the partial pressure of oxygen in the tissues to be increased, with a consequent improvement of the microcirculation of the organ.

Among suggested methods of improving the microcirculation of the intestinal graft, our attention was drawn to a report by Chernousov et al. [7] on the use of HBO for the prevention of graft necrosis. However, no reference could be found in the literature to any previous investigations aimed at quantitative assessment of the state of the microcirculation of an intestinal graft intended for esophagoplasty and treated with HBO.

The object of this experimental study was accordingly to examine the microcirculation in the muscular layer of a graft of small intestine designed for esophagoplasty, using HBO to correct any ischemic disturbances in the graft.

KEY WORDS: esophagoplasty; hyperbaric oxygenation; tissue blood flow.

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